STABILIZATION OF THE IMMUNOGENIC PROPERTIES OF THE GIRARD AND ROBIK EV STRAINS

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STABILIZATION OF THE IMMUNOGENIC PROPERTIES OF THE GIRARD AND ROBIK EV STRAINS

- USSR -

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Report III. Effect of Passage Through Animal Organism and Significance of Choice of Colonies on the Immunogenicity of the EV Strain.

The immunogenicity of vaccine strain is a variable property, therefore, the problem of stabilization of immunogenic qualities of vaccine strains is one of the urgent and most complex of problems in live vaccines.

Vaccine strains, like other strains subject to the general-biological law of variability, during storage on artificial nutrient media are gradually saprophytized, which is accompanied by change in their antigenic structure -- loss of the Vi- antigen or the VW antigens of Berrous and Bacon.

All known plague vaccine strains to some extent have lost their immunogenicity. The most stable has been the EV strain, although it at this time differs significantly from the subculture obtained in 1936 from the Girard strain.

To prevent vaccine strains from saprophytizing in general the same methods are recommended as are used in maintenance of the virulency of plague bacillus: storage at low temperature in sealed test tubes on blood or hormone agar, very infrequent reculturing, storage in the dessicated state . Dessication by the lyophilic method gives the best results of all the procedures available. However, repeated drying can lead to diminished immunogenic properties of the strain due

to accumulation of cells with reduced synthesizing capacities, which have become adapted to an anabiotic state. Therefore, we set ourselves the goal of studying the question of whether the immunogenic properties of the EV strain can be enhanced by passage through animal organisms. In addition, we attempted to investigate the immunogenicity of the cellular composition of the culture by studying individual colonies.

The EV strain (the NIIEG [*], dessicated in 1961), was passed through the guinea pig organism by two methods: 1) through the conjunctiva of the uncamaged eye, and 2) by intraperitonial and subcutaneous administration of the culture in small (decreasing) dosages.

In the conjunctiva passage a two-day old agar culture of the EV strain was applied on the conjunctival membrane in the amount of one loop (the bacilli were readily separated from the loop by emulsifying in the surface fluid of the eye). Usually this operation is not accompanied by visible reaction -- the eye remained unchanged, in some cases moderate hyperemia and conjunctivitus were noted. In 24 hours after inoculation with the platinum loop the detached eye was collected and seeded in agar plates containing blood and gentian violet. The culturing was done daily until negative results were obtained. Investigation showed that after two days the colonies from the agar plates increased less than at the moment of infection in the eye, and by the third day their number had risen somewhat and remained stationary until the sixth-seventh day, subsequently the colony number again began to decrease, and by the ninth-tenth day the cultures remained sterile.

Further, we determined the immunogenic properties of the EV strain for different durations of stay in the eye.

In the first experiment, we studied the immunogenicity of cultures previously passaged twice through the eye of guinea pig No 215 and removed on the third, fifth, and seventh day. Three groups of mice were inoculated with these three cultures (EV 215_3, 215_5, and 215_7). The original EV culture served as control In 21 days all the mice were inoculated with 200 Dcl of strain No 708.

We obtained the best results, compared with the original strain, with the strains No 215_5 and 215_7, while strain No 215_3 (Table 1) was less preserved in the animals.

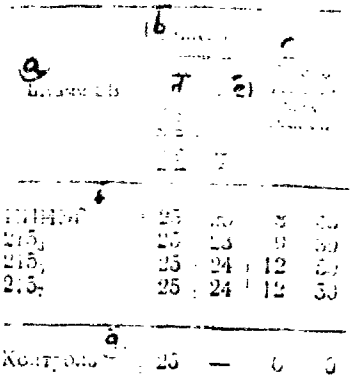
Based on the data, we can generally assume that on the fifth-sixth-seventh day of residence in the eye, the strain evidently became more active. After 5-7 days, the number of bacilli in the eye began to decrease. This duration coincided with the development of immunity.

Strain No 215_5 was passed three more times through guinea pig eyes (No 152, 159, and 160). Immunogenic properties of the culture isolated on the sixth day from the eye of guinea pig No 160 was studied by subcutaneous injection into the guinea pig of two-day old agar culture in the amount of 1000 microbial cells. At the same time, another group of guinea pigs were immunized with the same dosage of the

*NIIEG -- Nauchno-issledovatel'skiy Institut Epidemiologii i Gigiyeny; Scientific-Research Institute of Epidemiology and Hygiene

TABLE 1

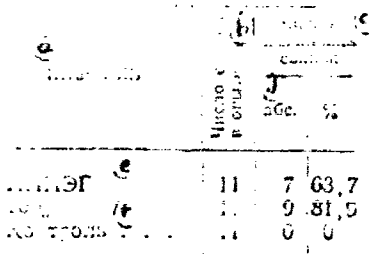
Immunogenic Properties of the EV Strain in Relation to Time of Stay in Guinea Pig Eye (inoculation dose of 500 bacilli, infection with 200 Dcl)



LEGEND: a) EV strain, b) number of mice; c) number of surviving mice; d) vaccinated; e) infected; f) NIIEG; g) control.

TABLE 2

Immunogenic Properties of the EV Strain After Passage Through Guinea Pig Conjunctiva (inoculation dose 1000 bacilli, infection with 200 Dcl)



Remark. Guinea pigs inoculated in the conjunctiva were then infected with 200 Dcl of virulent culture and all survived. LEGEND: a) EV strain; b) number of guinea pigs in experiment; c) number of surviving guinea pigs; d) absolute; e) NIIEG; f) control.

same dosage of the EV-NIIEG strain. After the fifth passage through the eyes of this strain immunogenic properties increased appreciably (Table 2). These experiments allow us to believe that the method of passage through the conjunctiva can be used to enhance the immunogenic properties of vaccine strains. Here we note especially the simplicity of the method.

In the subcutaneous and intraperitoneal passages, the EV strain was administered during the first five passages in the amount of 500 million bacilli, and in the remaining passages -- 200 million. Each method was used for nine passages. Upon the end of the passages the nonvirulence of the subculture was tested on guinea pigs. As was to be expected, all the animals survived. On the 30th day, they were sacrificed and their organs subjected to histological examination. Examination was made of the organs of guinea pigs infected with subcultures isolated after subcutaneous and intraparitoneal passages, and also with subcultures of the EV strain obtained after 12 passages through the guinea pig organism. Upon examination at the site of culture administration, hemorrhagic foci were found, along with a slight edema of the subcutaneous plexus and plethora. In the regional lymph

nodes pronounced plethora was also observed. Hyperplasia of the reticular cells, chiefly the sinuses of medullary substance was moderately pronounced and diffuse, only in individual cases were nodular growths of granulated tissue with small abscessing foci found. The spleen was moderately plethoric, its folliculi and pulp were slightly hyperplasic. Foci of interstitial pneumonia were found in the lungs. Signs of granular dystrophy and moderate plethora were discovered in the myocardium, liver, and kidneys. Histological studies afforded the conclusion that the EV vaccine strain persistently retained its original characteristics. After twelve-fold passage through the guinea pig organisms no changes were observed which would point to increased virulency and reactogenicity of the strain; its innocuousness also remained unchanged.

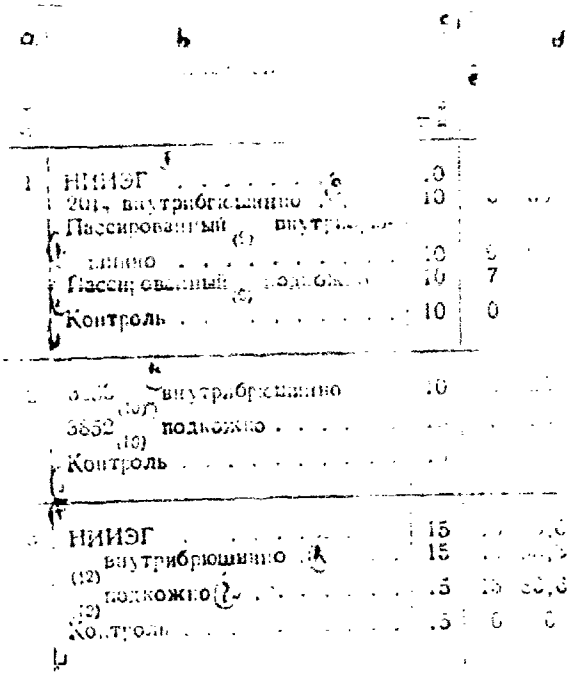
The immunogenicity of the passaged EV subculture in the dosage of 500 bacilli was tested on guinea pigs. Twenty days after vaccination the animals were subcutaneously infected with virulent culture. Through vaccination with the strains, and also with the strain No 201 (EV-201) isolated from guinea pig No 201, dying on the ninth day after intraperitoneal administration of 6 billion bacilli which had been passaged twice previously through the abdominal cavity of guinea pigs, it was established that the method of repeated passaging in small dosages through the abdominal cavity (Table 3) is the most promising method for retention and enhancement of immunogenic properties of a vaccine strain.

The EV strain thus isolated protected 90 % of the animals. An unpassaged EV subculture under the same experimental conditions resulted in the protection of only 60 % of the guinea pigs. In this case, intraperitoneal passages through the organism proved to be somewhat more effective than subcutaneous, therefore we decided once more to verify which method should be chosen for passaging which aims at enhancing the immunogenic properties of the vaccine strain. Both subcultures tested were passaged beforehand on the same rate in guinea pigs, who were administered subcutaneously and interperitoneally a two-day old agar culture in the amount of 200 million bacilli. On the sixth day the guinea pigs No 3852 and 3853 were sacrificed and the cultures removed from them -- EV-3852 (subcutaneous) and EV-3853 (intraperitoneal) -- were administered guinea pigs in the amount of 500 bacilli. In 21 days after the vaccination, the guinea pigs were infected subcutaneously with 200 Dcl of the virulent strain No 708.

In order to be convinced that the results were reliable, we made a third experiment to determine the effect on immunogenicity of the strain by passing through the organism. Analysis of data showed that passage through the organism is unquestionably a method affording enhanced immunogenic properties of the vaccine strain. However, in comparing the results of all three experiments, no advantage for any given method of passage was noted.

TABLE 3

Immunogenic Properties of the EV Strain After Being Passaged Through the Guinea Pig Organism (inoculation dosage 500 bacilli, infection with 200 Dcl)



LEGEND: a) number of experiment; b) EV-strain; c) number of guinea pigs in experiment; d) number of surviving guinea pigs; e) absolute; f) NIIEG; g) intraperitoneally; h) passaged (9) intraperitoneally; i) passaged (9) subcutaneously; j) control; k) intraperitoneally; l) subcutaneously.

During the studies we (together with Anisimova, Goncharova, and Karaseva) believed it possible to find out how prolonged stay of the EV strain in the organism affected the strain's immunogenic properties. Guinea pig No 405 among others was subcutaneously administered 100 bacilli of EV strain of the NIIEG-61 stock (unpassaged). On the 35th day, this guinea pig was sacrificed. Upon dissection, no visible pathologoanatomical changes could be detected in its organs, but plague bacillus cultures developed from seedings made with material taken from the inoculation site, regional lymph node, and spleen. The fact of the prolonged residence of the avirulent in the guinea pig organism posed the question as to whether this really was the EV strain or some other strain. In the laboratory where the study

was made, while there were no other glycerine-negative strains, still cultures isolated by seeding in peptone glycerine-containing broth evidenced, as to morphology of colony, growth in groth, and bacteriophage test, identity with the EV strain.

In order to discover what effect prolonged residence of the vaccine strain in a sensitive organism has on its immunogenic properties, we compared the protective properties of the EV strain of the NIIEG stock and the same strain residing for 35 days in the guinea pig organism (EV-405). Two inoculation doses were tested -- 100 and 500 bacilli of a two-day agar culture. In 21 days after the vaccination, the animals were infected with virulent culture. It was found that as a result of prolonged residence within the organism the immunogenic properties of the EV strain dropped sharply (Table 4). This effect can be explained by the fact that the EV strain was present in an immune organism, and this doubtless acted destructively on its immunogenic properties.

TABLE 4

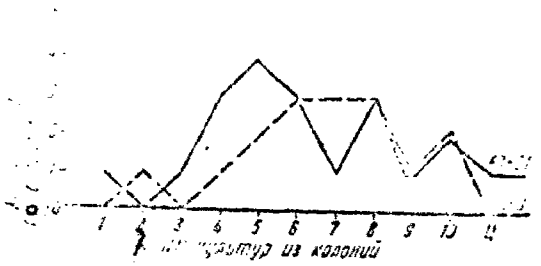
Effect of Prolonged (35 days) Stay of the EV Strain in the Guinea Pig Organism on its Immunogenicity (infection with 200 Dcl)

(a) ...ным ЕВ	(b) Доза вакцины (число микробов)	(c) Число свинок в опыте	(d) Число выживших свинок	
			(g) абс.	%
(e) ...	100	15	8	53,3
	500	15	10	67,5
	100	15	0	0
(f)	500	15	0	0
	—	15	0	0

LEGEND: a) EV strain; b) vaccine dosage (number of bacilli); c) number of guinea pigs in experiment; d) number of surviving guinea pigs; e) NIIEG; f) control; g) absolute.

It was established that a pure culture of pathogenic bacillus on synthetic nutrient media represents a population consisting of cells differing in virulence and antigenic properties. When selecting colonies in order to study immunogenic properties of intestinal-typhoid group bacilli colony morphology is usually used. When studying plague from such a point of view, difficulties are encountered (with rare exceptions when the strain clearly dissociates) due to uniformity of the colonies, especially the EV strain. Therefore, in this portion of the experiment, we used the following method.

A standard culture of EV strain dessicated at the Mikrob Institute in 1950 (EV-8) and a standard EV culture of NIIEG stock, dessicated in 1961, were dissolved in saline solution, one loop was seeded in five ml of broth and from the growing two-day culture one loop was seeded in a series of light touches [shtrikhi] on agar plates. In two days, typical plague bacilli had grown on the agar from both standard cultures. Under microscopic control, 10 colonies of each subculture were selected. Each colony was individually seeded in a test tube containing the varied [skoshennyy] agar and in a broth. In all, 20 colonies were seeded with this method. After two days of growth at 28° the test tubes containing cultures grown on the varied agar were sealed and placed in a refrigerator. Growth of the selective colonies in the broth was typical in all test gubes. Optically, the culture standard (after mixing) corresponded to 500 million bacilli. Only in one test tube seeded with the ninth culture of the EV-8 strain was the broth slightly turbid and exceeded the turbidity of the standard by one billion bacilli.



Immunogenic properties of cultures from individual colonies of the EV strain. LEGEND: a) number of surviving white mice; b) culture number of the colony.

Immunogenic properties were studied in mice. Broth culture of each colony was dissolved up to 10^{-4} for this purpose and from this dilution each mouse was administered 0.2 ml. This dose corresponded to approximately 10,000 bacilli. Immunogenic properties of each colony was studied in an approximate manner on 5 mice; in addition, two groups of 5 mice each were immunized, [one strain to a group] with the same dose of the whole culture of the EV-61 and EV-8 strains. A total of 110 mice were vaccinated, which were infected in 20 days with 200 Dcl of virulent culture. The experiment showed that individual colonies differed in their protective properties (Figure).

TABLE 5

Immunogenic Properties of Colonies of Two Subcultures of the EV Strain (inoculation dose: 10,000 bacilli, infection: 200 Dcl)

	4	22	19	86
	5	24	22	91
	8	22	21	95
		23	17	73
	Цель-	23	18	78
		25	0	0

LEGEND: a) strain; b) colony number; c) number of mice in experiment; d) number of surviving mice; e) absolute; f) EV-61; g) EV-8; h) EV-6.; i) control; j) whole.

For further investigation, we selected cultures of colonies which had provided the best indices of immunogenicity in the exploratory experiments, and verified these in a larger number of mice. Three colonies of the EV-61 strain (fourth, fifth, and eighth) and one of the EV-8 strain (tenth) were used. A two-day agar culture of each colony was administered to 25 mice in a dosage of 10,000 bacilli. After immunization two-three mice chosen at random were sacrificed from each group. The remaining animals were infected with 200 Dcl of virulent culture. Examination showed (Table 5) that cultures growing from individual colonies of the same strain can differ in immunogenic properties. Colonies of different immunological activity preserved wholly identical form and did not differ in biochemical or cultural characteristics, virulency, and reactogenicity.

Conclusion

1. Repeated passage through guinea pig conjunctiva or subcutaneous and intraperitoneal administration of diminishing doses of EV strain culture provided for enhancement and stabilization of the

immunogenicity of the strain. The passage methods used did not lead to more intense virulency and reactogenicity of the EV strain; experiments in animals and pathohistological investigations confirmed its initial innocuousness.

2. In undertaking passages a culture isolated from a guinea pig sacrificed not later than the fifth-sixth day after inoculation should be used.

3. Prolonged residence of the vaccine culture in the animal organism led to a significant decrease in its immunogenicity.

4. Cultures obtained from individual colonies of the same strain, not differing in form, is different in immunogenicity. The method of studying individual colonies can be employed for selecting immunogenicity of EV strain subcultures.

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